


Applicant: Hiroaki Shizuya
Application No.: 09/896,509
Filed: June 28, 2001
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In the Claims

Please enter claims 1, 2, 25, 43, 46, 47 and 55 amended to read, as follows:

1. (Amended) A method for identifying an essential chromosomal gene in a haploid test organism, said method comprising:



constructing a BAC-carrying merodiploid test cell by transforming a wild-type haploid host cell whose genome is known, which is capable of being transformed by artificial means and is capable of undergoing DNA recombination, with a bacterial artificial chromosome (BAC) carrying a known segment of DNA of the haploid test organism, which segment is homologous to a known segment of chromosomal DNA in the host cell, and wherein replication of the BAC in the test cell is sensitive to an environmental condition that selectively prevents replication of the BAC in the host cell;

inserting randomly a bacterial transposon into the merodiploid test cell so as to disrupt function of a gene therein;

culturing one or more of the BAC-carrying merodiploid test cells in a suitable culture medium while introducing the environmental condition so as to transform the merodiploid test cells into haploid test cells; and

identifying one or more of the haploid test cells that contain transposon-mutagenized DNA in an essential chromosomal gene therein.

2. (Amended) The method of claim 1 further comprising obtaining the essential chromosomal gene in the test cell by identifying, by sequence comparison with the known genome of the host organism, a gene in the known segment of DNA from the test organism that has been disrupted by the transposon.

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25. (Amended) A method for screening bacterial genes in a pathogenic bacterium whose genome is known to select compounds with putative antibiotic activity, said method comprising:

constructing a BAC-carrying merodiploid test cell by transforming a wild-type haploid host cell whose genome is known, which is capable of being transformed by artificial means and undergoing DNA recombination, with a BAC that carries a known segment of DNA of a pathogenic bacterium, and wherein the BAC in the test cell is sensitive to an environmental condition that selectively prevents replication of the BAC in the test cell;

inserting randomly a transposon into the merodiploid test cell so as to disrupt function of a gene therein;

culturing one or more of the merodiploid test cells in a suitable culture medium while introducing the environmental condition;

identifying one or more test cells that do not survive subjection to the environmental condition as containing the transposon in an essential chromosomal gene therein;

obtaining the essential gene in the known segment of DNA of the pathogenic bacterium by identifying, by sequence comparison with the known genome of the host cell, a gene in the known segment of DNA that has been disrupted by the transposon; and

screening the essential gene obtained from the pathogenic bacterium or a bacterial protein encoded by the corresponding essential gene against putative antibiotic compounds to determine those compounds that bind to or interrupt function of the essential gene or the bacterial protein, wherein such a compound is a candidate antibiotic against the pathogenic bacterium.

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43. (Amended) A method for identifying an essential chromosomal gene in a haploid test organism, said method comprising:

34 constructing a BAC carrying a known segment of DNA of the haploid test organism, which segment is homologous to a known segment of chromosomal DNA in a haploid host cell having a known genome, which is capable of being transformed by artificial means and is undergoing DNA recombination;

inserting randomly a bacterial transposon into the BAC so as to disrupt function of a gene in the segment of chromosomal DNA;

introducing the BAC into the a haploid host cell to create a merodiploid test cell;

culturing the merodiploid test cell in a suitable culture medium such that the BAC in the test cell is sensitive to an environmental condition that selectively prevents replication of the BAC in the test cell;

identifying one or more BAC-carrying merodiploid test cells that do not survive in culture as containing the transposon in an essential chromosomal gene therein; and

obtaining the identity of the essential chromosomal gene by identifying a gene that has been disrupted by the transposon by sequence comparison between the known genome of the host cell and the known segment of DNA inserted into the BAC.

35 46. (Amended) The method of claim 43, wherein the transposon is inserted randomly into the BAC *in vitro* prior to introduction of the BAC into the test cell.

47. (Amended) The method of claim 46, wherein the known segment of DNA is linearized prior to introduction into the test cell.

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55. (Amended) The method of claim 54, wherein the method further comprises screening a
essential gene obtained from the pathogenic bacterium or a bacterial protein encoded thereby
against putative antibiotic compounds to determine those compounds that bind to or interrupt
function of the corresponding essential gene or the bacterial protein, wherein such a compound is
a candidate antibiotic against the pathogenic bacterium.